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Combined use of metformin and ethinyl estradiol–cyproterone acetate in polycystic ovary syndrome

Mitkov M.[a], Pehlivanov B.[b,*], Terzieva D.[c]

[a]*Department of Endocrinology, Medical University, Plovdiv, Bulgaria*
[b]*Department of Obstetrics and Gynecology, Medical University, 15A Vassil Aprilov Blvd., 4000 Plovdiv, Bulgaria*
[c]*Central Clinical Laboratory, Medical University, Plovdiv, Bulgaria*

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Abstract

Objective: The aim of our study was to compare the effect of metformin applied independently to the effect of metformin used in combination with oral contraceptive containing ethinyl estradiol (EE) and cyproterone acetate (CA).
Study design: This prospective, open clinical study lasted 6 months and included 30 women with PCOS, divided in two groups of 15 women each. Group 1 received 850 mg metformin twice a day and group 2 in which Diane35 was added to the same treatment only during the first 2 months of the investigation. Serum levels of testosterone, immune reactive insulin (IRI), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) and lipid metabolism parameters were measured before the treatment, on the third and sixth month. Free androgen index (FAI) and HOMA-IR were calculated. Body mass index (BMI) and waist-to-hip ratio (WHR) were assessed at baseline and at the end of therapy.
Results: Much better and faster decrease in the level of testosterone and free androgen index in group with combined use of metformin and Diane35 was established, without deterioration of the anthropometric and biochemical indices and insulin sensitivity.
Conclusion: The combination of metformin with intermittent application of Diane35 is an appropriate alternative for the pathogenic influence and clinical improvement of the symptoms of androgen excess in cases with PCOS.

Keywords: PCOS; Metformin; Oral contraceptive; Insulin resistance

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies, affecting 5–10% of women of reproductive age [1]. It is a heterogenic collection of symptoms with genetic predisposition, which combined in a different way, determine the variety of clinical appearance. Some of the patients have no complaints whatsoever and the clinical signs are hardly noticeable. Other patients show expressive reproductive, hormonal and metabolic deviations. In clinical practice, women with PCOS are seen for three major reasons: infertility (mean incidence 74%), menstrual irregularity (mean incidence of dysfunctional bleeding 29%, mean incidence of amenorrhea 51%), and androgen excess (mean incidence of hirsutism 69%) [2].

The prevailing current opinion is that the main cause for all these symptoms is hyperinsulinaemia, determined by existing insulin resistance [3]. However, since the underlying defect in PCOS is insulin resistance, insulin-sensitizing drugs have emerged as new therapies. These drugs have the potential to manage the entire spectrum of reproductive, endocrine, and metabolic abnormalities associated with PCOS [4]. There are two classes of insulin-sensitizing drugs currently available, namely the biguanide metformin and the thiazolidinediones, which include troglitazone (not currently in use because of serious adverse effects), rosiglitazone and pioglitazone [5].

The most commonly used drug of the kind is metformin [4–6]. In PCOS patients, the majority of studies show that metformin decreases insulin resistance and some endocrine

* Corresponding author. Tel.: +359 32 955518; fax: +359 32 626383.
E-mail address: blago1956@yahoo.com (B. Pehlivanov).

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and metabolic parameters—it reduces the level of insulin, testosterone and LH concentrations, which are usually elevated in these patients [4,6–8]. In most studies, however, optimal results are to be expected after at least 6 months. That fact has suggested a possibility for enhancing the effect of metformin by adding another drug, which would lead to better and faster normalization of the disturbed endocrine parameters.

The aim of our study was to compare the effect of metformin, used independently and in combination with ethinyl estradiol (EE)–cyproterone acetate (CA) pill, applied only during the first 2 months of the therapy.

2. Materials and methods

The study is prospective, including 30 women aged 19–29 (mean 23.8 ± 2.8), matching the current criteria for PCOS-, oligo- or anovulation, clinical and biochemical signs of hyperandrogenism and polycystic ovaries. The diagnosis was made in the presence of two out of the three mentioned criteria [9], and in our study, hormonal hyperandrogenism was an absolute condition. Additionally included criteria were the level of immune reactive insulin (IRI) over 12.0 IU/l and homeostasis model assessment of insulin resistance (HOMA-IR) over 2.75. All subjects were euthyroid, and adrenal hyperplasia and hyperprolactinemia were ruled out. After calculating the body mass index (BMI) and waist-to-hip ratio (WHR) of the enrolled patients, the following tests were made: IRI, total testosterone (*T*), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TG).

The patients were divided into two groups of 15 women each, similar in age and BMI. The first group (Group 1) received metformin (Metfogamma® 850, Woerwag Pharma GmbH & Co.) 850 mg, p.o. b.i.d. with meals. The second group (Group 2) was treated with the same dose of metformin combined with Diane35® (35 μg EE, 2 mg CA, Schering AG, Berlin, Germany) applied only during the first 2 months. The above-mentioned clinical, hormonal and metabolic parameters were followed at the end of the third and sixth month. All laboratory tests were performed in the early follicular phase of spontaneous bleeding or withdrawal bleeding. The hormonal tests were made on AxSYM™ SYSTEM (Abbot Diagnostics). Immune reactive insulin (IRI, normal values 5–20 IU/l) was determined using microparticle enzymatic immunoassay (MEIA). Testosterone (normal values 0.1–1.2 ng/ml), SHBG (normal values 15–120 nmol/l) and DHEAS (normal values 0.8–3.9 μg/ml) was determined using enzyme-linked immunosorbent assays (ELISA). Free androgen index (FAI) was calculated as 100 × *T*/SHBG and HOMA-IR was calculated as IRI × blood glucose/22.5. In order to unify the measurement units, the values for testosterone were converted from ng/ml to nmol/l using the following index (proposed by manufacturer) 1 ng/ml = 3.467 nmol/l. Serum levels of total cholesterol, triglycerides and HDL-cholesterol were determined by standard methods. BMI and WHR were calculated by the commonly used formula.

The average intra-assay coefficients of variation (CV) were 5.82% for testosterone, 6.5% for DHEAS, 8.6% for SHBG and 4.1% for IRI.

The data was analyzed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). The distribution of the data was tested using the Kolmogorov–Smirnov test for normality. The mean and the SEM were calculated for all parameters. Comparison between groups was performed by an unpaired *t*-test for normally distributed data. Results were considered significant if $P < 0.05$. All *P*-values are two-tailed.

The study was approved by the Ethics Committee of the Medical University of Plovdiv, Bulgaria, and informed written consent was obtained from each subject.

Table 1
Clinical, endocrine and metabolic parameters of the subjects before and during treatment

Parameters (mean ± S.E.)	Metformin			Metformin ± Diane35®		
	0 month	3 month	6 month	0 month	3 month	6 month
BMI (kg/m^2)	27.9 ± 1.18	27.4 ± 0.98	26.9 ± 0.89	27.8 ± 0.77	27.6 ± 1.00	27.3 ± 0.53
WHR	0.79 ± 0.02	0.80 ± 0.02	0.79 ± 0.01	0.80 ± 0.01	0.80 ± 0.02	0.79 ± 0.02
Testosterone (ng/ml)	2.05 ± 0.03	1.85 ± 0.06**	1.60 ± 0.03***	2.13 ± 0.05	1.45 ± 0.03***	1.31 ± 0.03**
IRI (μU/ml)	17.08 ± 1.38	14.07 ± 0.26*	11.84 ± 0.37***	15.13 ± 0.43	12.22 ± 0.39***	9.01 ± 0.26***
SHBG (nmol/l)	31.81 ± 2.11	33.03 ± 1.62	37.12 ± 1.31$^{\#}$	30.62 ± 1.67	37.25 ± 1.63**	38.84 ± 1.42
FAI	23.66 ± 1.54	20.13 ± 1.14	15.49 ± 0.54***	24.99 ± 1.23	13.84 ± 0.51***	12.21 ± 0.61*
DHEAS (μg/ml)	2.69 ± 0.19	2.42 ± 0.21	2.36 ± 0.14	2.33 ± 0.17	2.30 ± 0.15	2.22 ± 0.13
HOMA-IR	3.48 ± 0.41	3.01 ± 0.07	2.47 ± 0.24$^{\#}$	3.55 ± 0.59	2.69 ± 0.32	1.96 ± 0.21***
TG (mmol/l)	1.54 ± 0.15	1.50 ± 0.1	1.29 ± 0.08	1.41 ± 0.14	1.33 ± 0.08	1.32 ± 0.07
Cholesterol (mmol/l)	5.22 ± 0.17	5.20 ± 0.17	5.14 ± 0.13	5.14 ± 0.13	5.20 ± 0.16	4.81 ± 0.14
HDL-C (mmol/l)	1.61 ± 0.08	1.61 ± 0.06	1.62 ± 0.05	1.49 ± 0.06	1.48 ± 0.04	1.48 ± 0.07

Values are mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the previous values; $^{\#}P < 0.05$ compared with baseline values.

3. Results

The results of our investigation are presented in Table 1. At baseline, all clinical, hormonal and metabolic parameters for both groups are similar. The better treatment of testosteronaemia in the group with combined application of metformin and Diane35 is obvious. The mean difference of testosterone in the sixth month compared with baseline values in group 1 is −0.45 (95% CI −0.54, −0.36), while in group 2 it is −0.82 (95% CI −0.94, −0.70). The difference in diminution is extremely significant ($P < 0.001$).

The decrease of testosterone in the third month in group 1 is 10%, while in group 2 it is 32%. In both groups, the decrease between third and sixth month is from 10 to 12%. The results clearly show that the addition of Diane35 at the beginning of the treatment leads to faster decrease in testosterone level. The changes we reported for SHBG are also of great significance. In group 1, although the absolute values increased, a statistically significant difference is barely reached during the sixth month. In group 2, however, a very significant increase in SHBG was reported in the third month, and an extremely significant—in the sixth month. It is the absence of significant changes between the third and sixth month that shows the role of Diane35 on SHGB level.

Important changes were achieved for FAI as well. In group 1, we reported no significant changes in the third month and extremely significant in the sixth month, compared to the basic values. In group 2 extremely significant changes compared to the starting values have been reported in the third, as well as in the sixth month (Fig. 1). The addition of Diane35 extremely diminishes the level of biologically active testosterone.

The selection of the subjects included in our study allowed us to estimate the effect of this treatment scheme in patients with outlined insulin resistance. The decrease in the values of IRI and HOMA-IR in both groups is significant during all stages of investigation. This proves that when combined with metformin, Diane35 not only avoids hyperinsulinaemia, but most probably leads to improvement in insulin sensitivity through greater decrease of androgens.

The changes in BMI for both groups are not significant, despite the decrease in their absolute values. The same concerns WHR, cholesterol and triglycerides. This gives us a solid ground to assert that our scheme is appropriate for application in patients with metabolic syndrome.

No serious adverse effects were registered throughout the whole treatment. Five women (33%) from group 1 and six women (40%) from group 2 reported dyspeptic symptoms at the end of the first month, which disappeared spontaneously.

4. Discussion

The current investigation concerning the metabolic consequences of PCOS shows that the optimal treatment aims to manage hyperandrogenism and at the same time to improve both insulin sensitivity and dyslipidemia.

The applying of several drugs in conjunction in the therapy of PCOS is not a novelty in world practice [10,11]. Most of the well-known schemes, however, show a negative effect that goes along with the positive influence on certain symptoms. We tried to combine the current pathogenic treatment with metformin with a short-term application of Diane35—a powerful antiandrogenic drug, traditionally used especially in Europe. A similar scheme has been offered by Elter et al. [11], but the difference in their treatment is that the application of Diane35 is continuous and therefore deprives the patients of the opportunity to

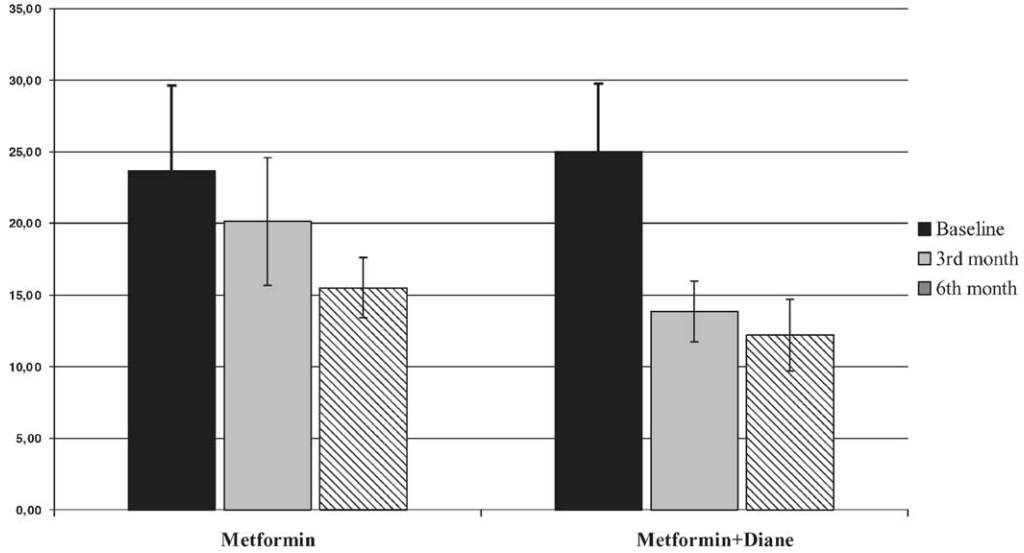

Fig. 1. Changes in FAI (mean ± S.D.) during metformin and metformin + Diane35 treatment.

conceive. Furthermore, the authors apply metformin in order to diminish the negative effect of OC on insulin sensitivity and the lipid profile. In contrast, our aim is to accelerate the effect of metformin with regard to hyperandrogenaemia.

Metformin is a biguanide that enhances peripheral tissue sensitivity to insulin and inhibits hepatic gluconeogenesis while increasing the uptake and utilization of glucose by muscles. By increasing insulin sensitivity, metformin reduces insulin resistance, insulin secretion, and hyperinsulinemia. It is antihyperglycemic in action and does not cause hypoglycemia [4,12]. The drug shows significant positive effects on lipid metabolism and the coagulation factors. There is no unanimity concerning the question whether metformin increases or decreases the level of SHBG. Most authors register increase of SHBG while others assert the opposite. According to Morin-Papunen et al. [13,14], the use of metformin lowers the circulating concentrations of testosterone. At the same time, it leads to a decrease in SHGB concentrations during therapy, which results that FAI did not change. This fact could explain why, despite the significant improvement in hyperandrogenism, the clinical result is not good [13–15]. In our study in the sixth month of treatment with metformin, the level of SHBG is significantly higher compared to the baseline values. In the group with combined application of metformin and EE + CA, the level of SHBG is significantly higher than that of baseline much earlier—in the third month. This result is expected having given the effect of EE upon the level of SHBG. The changes in the level of SHBG affect the values of FAI as well. In group 2, the index is much higher than the initial values, whereas in cases of monotherapy with M, FAI increases more slowly. Our study confirms that metformin therapy results in reduction of hyperinsulinemia with a parallel decrease of testosterone level.

The monotherapy with combined oral contraceptives is considered a treatment of choice when in cases of women, who do not want to conceive [6]. These drugs decrease the level of total testosterone and increase SHBG, which results in diminishing the level of biologically active testosterone. The oral contraceptive we used—Diane35, has CA as progestogen. Cyproterone acetate is a potent progestagen and antiandrogen. It blocks the binding of dihydrotestosterone to its receptor, reduces 5-α reductase activity and impairs androgen steroidogenesis [16]. PCOS, however, is often combined with overweight, dislipidemy, hyperinsulinaemia and the application of OC in such cases is not always recommended [17]. Women with PCOS are already at high risk for type 2 diabetes and OC use might be expected to increase the relative risk for this even more [18]. Contraceptives might aggravate insulin resistance and induce glucose intolerance, which has been reported in several studies. The study of Dahlgren et al. [19] demonstrated significant deteriorations of insulin sensitivity in women with PCOS treated with a high-dose OC containing cyproterone acetate administered for 6 months. In our study, the decrease of IRI and HOMA-IR in the group with combined application of metformin and Diane35 proves that the short-term application of EE + CA does not impair insulin sensitivity. The study by Korytkowski et al. [20] also assessed a control group of normal women, in whom OC administration was associated with an increase in serum triglycerides—a finding consistent with the induction of insulin resistance. In our study, the addition of EE–CA to metformin did not cause a change in the lipid profile, probably due to its short time of application.

No changes in BMI are registered at the beginning and at the end of the investigation in both groups. This leads to the conclusion that the combination of metformin and EE + CA does not result in increase of body weight. That makes possible the combined usage of these drugs in cases concerning overweight patients. The application of both drugs according to our scheme has several advantages—a rapid decrease in the level of free testosterone, an absence of impairment of insulin sensitivity and lipid balance, a compensation of the negative effect of Diane35 on body weight, and a limitation in conception for a comparatively short period of time.

The short duration of our investigation does not allow us to make conclusions concerning the changes in several clinical symptoms such as hirsutism, overweight and regulation of the menstrual cycle. The assessment of hirsutism is relatively subjective [9] and the latter is often treated by women themselves (mostly by mechanical methods of removal or non-pharmacological ones). Moreover, the necessary period of time for clinical improvement of hirsutism is usually longer than 6 months. For that reason, we decided not to mention the evaluation of this clinical sign. The reported changes in the basic hormonal parameters, however, make the improvement of clinical signs quite logical and expected. This fact makes us consider the necessity of longer duration of treatment and possible intermittent courses with Diane35, depending on the clinical and hormonal results.

5. Conclusion

The treatment of PCOS requires an individual approach according to the primary complaints of the patients and the leading clinical, hormonal and biochemical disturbances. The results of our study give us a solid ground to affirm that the combined usage of metformin and EE + CA is much more appropriate as initial therapy of PCOS than metformin alone. We presume that the intermittent application of Diane35 is recommended in cases of patients poorly responding to metformin monotherapy. A similar treatment should be applied to a greater number of patients, so that a better classification of clinical and hormonal signs is achieved. In this way, the indications for using our scheme of treatment will be determined much better.

References

[1] Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853–61.

[2] Guzick D. Polycystic ovary syndrome: symptomatology, pathophysiology, and epidemiology. Am J Obstet Gynecol 1998;179:S89–93.

[3] Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanisms and implications for pathogenesis. Endocrine Rev 1997;18:774–800.

[4] Baillargeon JP, Iuorno MJ, Nestler JE. Insulin sensitizers for polycystic ovary syndrome. Clin Obstet Gynecol 2003;46:325–40.

[5] Velazquez EM, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 1997;90:392–5.

[6] Tan WC, Yap C, Tan AS. Clinical management of PCOS. Acta Obstet Gynecol Scand 2001;80:689–96.

[7] Moghetti P, Castello R, Negri C, et al. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized double-blind, placebo-controlled 6-month trial followed by open, long-term clinical evaluation. J Clin Endocrinol Metab 2000;85:139–46.

[8] Lord JM, Flight IH, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003;327: 951–5.

[9] The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome, Fertil Steril 2004;81:19–25.

[10] Ibáñez L, Zegher F. Low-dose combination of flutamide, metformin and an oral contraceptive for non-obese, young women with polycystic ovary syndrome. Human Reprod 2003;18:57–60.

[11] Elter K, Imir G, Durmusoglu F. Clinical endocrine and metabolic effects of metformin added to ethinyl estradiol–cyproterone acetate in non-obese women with polycystic ovary syndrome: a randomized controlled study. Human Reprod 2002;17:1729–37.

[12] Kirpichnikov D, McFarlane S, Sowers J. Metformin: an update. Ann Int Med 2002;137:25–33.

[13] Morin-Papunen LC, Vauhkonen I, Koivunen R, Ruokonen A, Martikainen H, Tapanainen JS. Metformin versus ethinyl estradiol–cyproterone acetate in the treatment of nonobese women with polycystic ovary syndrome: a randomized study. J Clin Endocrinol Metab 2003;88:148–56.

[14] Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Martikainen HK, Tapanainen JS. Endocrine and metabolic effects of metformin versus ethinyl estradiol–cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study. J Clin Endocrinol Metab 2000;85:3161–8.

[15] Stadtmauer LA, Wong BC, Oehninger S. Should patients with polycystic ovary syndrome be treated with metformin? Human Reprod 2002;17:3016–25.

[16] Fruzetti F. Treatment of hirsutism Antiandrogen and 5-α-reductase inhibitor therapy. In: Azziz R, Nestler JE, Dewailly D, editors. Androgen Excess Disorders in Women. Philadephia: Lippincott/ Raven; 1997. p. 787–97.

[17] Nestler JE. Should patients with polycyctic ovary syndrome be treated with metformin? An enthusiastic endorsement Human Reprod 2002;17:1950–3.

[18] Diamanti-Kandarakis E, Baillargeon JP, Iuorno MJ, Jakubowicz DJ, Nestler JE. A modern medical quandary: polycystic ovary syndrome, insulin resistance, and oral contraceptive pills. J Clin Endocrinol Metab 2003;88:1927–32.

[19] Dahlgren E, Landin K, Krotkiewski M, Holm G, Janson PO. Effects of two antiandrogen treatments on hirsutism and insulin sensitivity in women with polycystic ovary syndrome. Hum Reprod 1998;13: 2706–11.

[20] Korytkowski MT, Mokan M, Horwitz MJ, Berga SL. Metabolic effects of oral contraceptives in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1995;80:3327–34.